Protein production in E. coli

Introduction

Based on a lab course (Snehadri).

Materials

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  - LB media
  - 50 mg/ml kanamycin stock
  - 1 M IPTG stock

Procedure

Production

1. Pick a single colony of each construct from the LB-kanamycin agar plate and inoculate a culture tube containing 5 ml LB-kanamycin medium. This tube will be used as a starter culture and is incubated overnight at +37C. Make an identical starter culture (from same colony) as reserve.

2. Transfer 1-2 ml of grown culture from starter culture tube to a 250 mL Erlenmeyer flask with 50 mL of LB-kanamycin medium and incubate at +37C shaking for 2-3 hours.

3. Transfer your 30 ml culture to inoculate a 1 L Erlenmeyer shake flask containing 200 mL LB-kanamycin medium. Incubate the shake flask at +37C for ~2 h (until A600 is 0.6-0.8). Measure the OD at 30 minute intervals and use clean LB-kan as blank.

4. Take a 500 ul sample from the culture for SDS-PAGE. Centrifuge the sample at 10000g for 2 min in an eppendorf centrifuge. Discard supernatant and suspend the pellet in 50 ul 2x SDS-PAGE sample buffer. Store at -20C until the SDS-PAGE is run.

5. Add 230 ul IPTG (from 1 M stock) to induce protein expression. This will give a final concentration of 1 mM IPTG. Allow the cultures to incubate for 4 hours at +37C shaking. Alternatively you can incubate culture after induction overnight at +30C.

6. Take a 500 ul sample from the culture for SDS-PAGE analysis. Harvest the remaining cells by centrifuging at 3200g for 12 min in swing bucket rotor at +4C in 50 ml falcon tubes. Use two tubes for every construct. You will need several rounds of centrifugation. Every time discard supernatant and load tubes with the next portion of culture up to the top. Discard the last portion of growth medium supernatant and store bacterial pellets at -20C until further use.